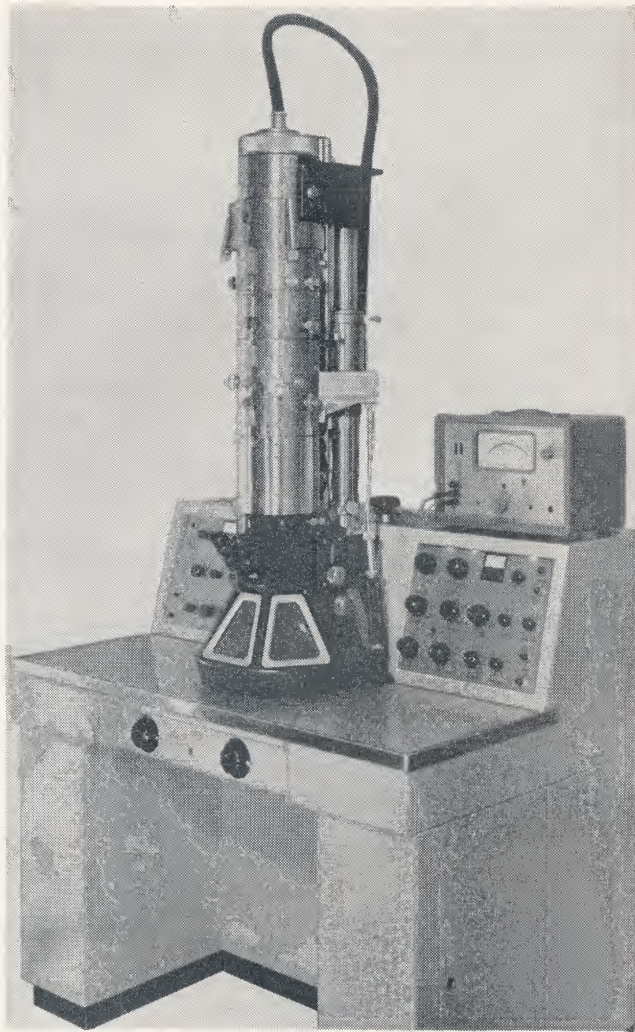


Instrumentation for Bio/Medical Research

by Vern W. Palen

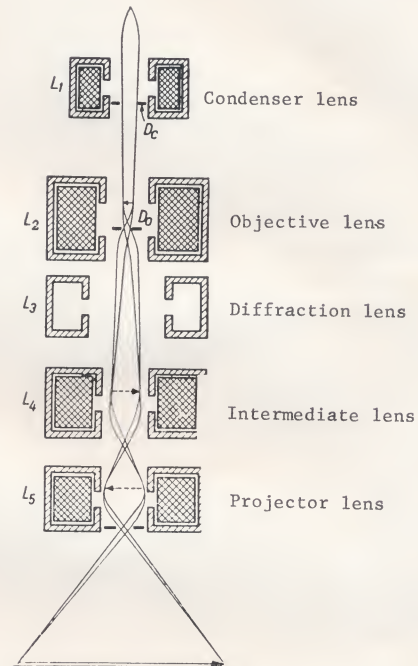


Electron Microscope

BIO-MEDICAL instrumentation techniques today include: Electron Microscopy, Contact Microradiography, Projection Microradiography (X-ray Microscopy), Electron Beam Microanalysis, X-ray Diffraction, and X-ray Spectrography. Assuming some readers may not be familiar with all of the named methods, it is well to preface the discussion with a brief explanation of the operating principle which underlies the design and application of each instrument used in the above methods.

The Electron Microscope essentially consists of an electron source (heated tungsten filament) and a series of magnetic lenses enclosed in an evacuated chamber. A condenser lens serves to focus the electron beam on the specimen which is mounted in the form of a thin film. Some electrons pass through the specimen, others are scattered and are lost. The resultant image is magnified by the objective, intermediate and projector lenses. This highly magnified image is projected onto a fluorescent screen or camera film. Most electron microscopes provide a resolving power of approximately 10 Angstroms.

Contact Microradiography requires a source of soft x-rays (5KV or less) to provide a fine focus beam. Specimen and film are placed in direct contact at some distance from the source. The recorded image is photo-



graphically enlarged later. Resolving power is about one micron. Contact Microradiography permits imaging of internal detail of specimens which are too thick for light microscopes or electron microscopes.

Projection Microradiography, often called X-Ray Microscopy, utilizes an electron beam, focused to a fine point, which strikes a metal target. X-rays generated at the point source in the target material are dispersed in a conical configuration. The specimen is placed near the apex of the cone and the film is at a greater distance from the x-ray source. Magnification is determined by the relationships in distance between source, specimen, and film. Target material may be changed to select different radiation wavelengths. This facilitates qualitative chemical analysis in micro areas. Resolution is about one micron.

Electron Beam Microanalysis projects electrons onto a specimen in order to generate characteristic x-rays from a very small area (about one micron). Secondary rays coming from the specimen are systematically diffracted by an analyzing crystal to a radiation detector. Electronic analyzing circuits provide readings of characteristic x-ray wavelengths and intensities. From this information, elements are identified. This method permits both qualitative and quantitative analysis of specimen areas approximately one micron in size.

X-Ray Diffraction, or Diffractometry, uses x-rays to bombard a specimen, then either records secondary diffracted rays on a camera film, or on a strip chart by means of a detector and electronic analyzing circuits. Basic components and x-ray optics of medical x-ray diffraction units are the same as those used by industry. However, the design is altered somewhat in order to accommodate medical and biological specimens. Diffraction and Diffractometry give qualitative and quantitative determinations on chemical compounds. This method is the only one that can handle the determination of crystal structures.

X-Ray Spectrography (fluorescence) bombards a specimen with x-rays; secondary emitted rays strike an analyzing crystal and then a detector. Pulses are recorded on a strip chart with the aid of an electronic analyzer. X-Ray Spectrography provides qualitative and quantitative information concerning elemental composition, from atomic #5 (boron) upward.

In biology, the electron microscopist uses a specimen of a once-living material which has been modified by chemical treatment, impregnated with plastics, sectioned with a glass or diamond knife, and which is finally bombarded with electrons. Brachet¹ discusses his work and the high degree of molecular organization of these fundamental particles of life. He recommends that bio-chemical techniques be integrated with morphological studies.

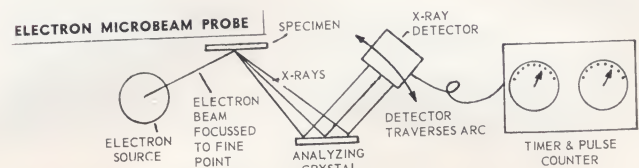
Green and Hatefi² have studied mitochondria and their equivalents as the energy transducers in aerobic organisms. They point out that structure and function of the mitochondrial system are intertwined, and also that for an understanding of enzymes, a precise bio-chemical description of structure is required. Chandra³ suggests a new concept of the mitochondrial membrane structure to explain observed events.

The ultrastructure of a unique type of cell inclusion was resolved by van Breeman and Montgomery⁴ in studies of renal proximal tubule cells. They use the term metasome, or exchange body, to designate three distinct morphological types. Like the industrial microscopist, the biologist does well to use a variety of microscopes and instrumentation to investigate his problem.

Bereczky, et al.,⁵ studied the sequence of changes in polyoma-infected mouse embryo cells by light, phase contrast, fluorescence, and electron microscopy. They were able to characterize two types of inclusions that developed in different cell nuclei, and to determine the effect on cell structure. Staining techniques and the significance of the combined observations of the chemical nature of the polyoma virus are also discussed.

The relationship between cell structure, as depicted by the electron microscope, and protein biosynthesis is covered by Campbell⁶. His work shows how a variety of techniques and efforts by two teams of biochemists can achieve a better understanding of protein synthesis complexities. His studies permit the development of a hypothesis as to where the proteins are synthesized and what structures are involved in their export from the cell.

Moscona⁷ has dispersed the cells of tissues and then studied how the cells recombined. He shows one electron micrograph that reveals a special device that some



Electron Probe Analyzer for identification of elements

cells may have for mutual attachment across their membranes. Stewart and Stewart⁸ reported on plasma membrane formation in the slime molds, *Myxomycetes*. Their hypothesis is that whenever a plasmodium needs a new plasma membrane, it uses the already formed membrane of the cytoplasmic vesicles.

Horne and Wildy⁹ review animal virus particles. Most viruses have helical or cubic symmetry. A number of viruses have been found to have 5:3:2 cubic symmetry with icosahedral structure. In particular, all adenoviruses examined to date by negative staining have a consistent structure of this type.

Anderson, et al.,¹⁰ has studied the agglutination of phages and polio viruses by antibody molecules. High resolution micrographs showed that thin fibers are attached to the surface of the particles, forming connecting bridges and at the same time keeping these particles from touching each other. Kaesberg¹¹ discusses structural information on the tobacco mosaic virus and other plant viruses. Electron microscopy, utilizing negative and positive staining techniques, has shown these particles to be hollow protein shells.

Considerable work has been done on the direct visualization of RNA and DNA molecules. Hall,¹² using the shadow-casting process, Kleinschmidt, et al.,¹³ using thin sections and a Langmuir trough preparation, and Beer and Zobel,¹⁴ using staining techniques, studied the size, shape, and morphological feature of DNA. Chatterjee and Sadhukhan¹⁵ directly visualized the dissociation of twin-standard DNA molecules which were

denatured by aging in an alkaline buffer. Recent refinements in techniques developed specifically for biological materials were reviewed by Howatson.¹⁶

Combining the techniques of polarization, fluorescence, phase, interference, and electron microscopy, Wolken¹⁷ has summarized his studies on plant and animal photoreceptors. The structure of ferritin has been confirmed by electron microscopy. Kurtz¹⁸ has resolved the predicted iron cores surrounded by a 100 Angstrom diameter protein shell. The use of well defined shape and geometric symmetry as a criterion of crystallinity was applied by Bettelheim and Philpott¹⁹ to three fractions of calcium chondroitin sulphate from bovine trachea.

In a comprehensive article, Gross²⁰ describes the structure of native and reconstituted collagen. Illustrations of high resolution micrographs and schematic drawings show the helical formation of the tropocollagen molecules. Gross also shows the manner in which they are arranged to produce segmentations typical of native and reconstituted collagen.

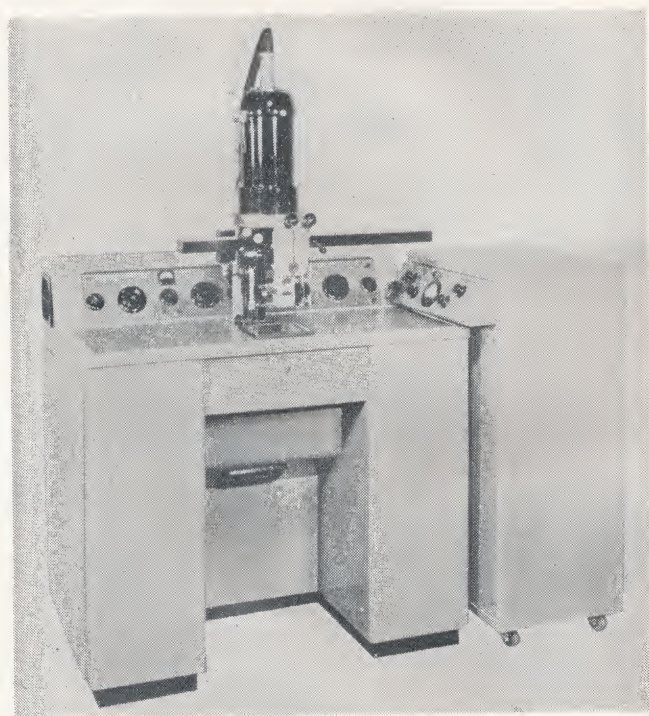
Electron diffraction, or diffracted beam electron microscopy, has gained renewed popularity. Here, the objective aperture is used to select a region of the diffraction pattern. The instrument is afterward returned to operation as a microscope. This technique can be used to identify, observe, and measure the size and distribution of selected particles adhering to a surface. Because of the limited exposure time available before the electron beam cross-links a polymer crystal, Niegisch and Swan,²¹ after recording the diffraction pattern, simultaneously magnified each of the spots by underfocusing the intermediate lens. Though this composite micrograph is not as clear as can be obtained by imaging a single diffraction spot, the crystal planes can be visualized, provided the crystal is of simple morphology.

X-ray and electron microscope techniques have been combined in a few instances. Asunmaa and Pattee,²² working at the Stanford University biophysics laboratory, have developed a technique for extending the resolution of microradiographs down to 600 Angstroms. The advantages of the microradiographic method as an adjunct to electron microscopy is described by Scott, et al.,²³ in their investigations of the mechanism of calcification in organic tissue. Van Huysen used the contact microradiographic method to resolve the structure of young dentine.²⁴

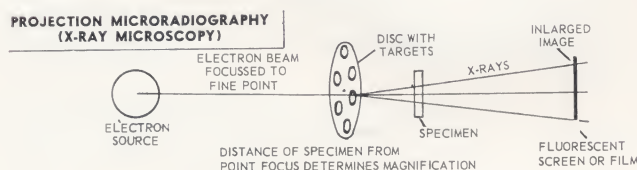
Projection X-Ray microscopy has been given new prominence by Ong²⁵ and Scott, who rely on the large depth of field, the wide choice of essentially monochromatic radiation and the direct magnification of the object to show the three-dimensional distribution of selected elements in a complex specimen.

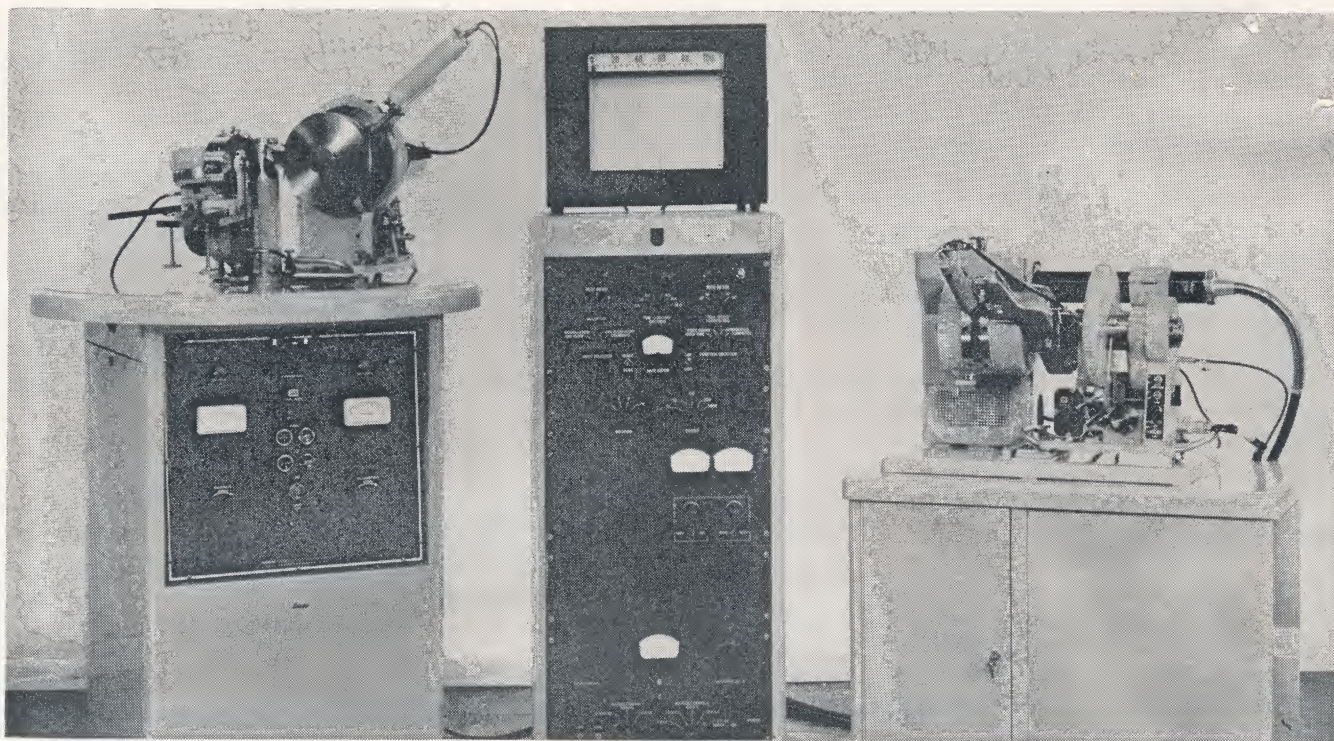
Stains are now being used for more than contrast enhancement. Bullivant and Hotchin²⁶ use chromyl chloride to stain thin sections of pancreas, cardiac muscle, etc. Besides contrast, the treatment selectively removes some components such as mucuous and zymogen granules and the core material of virus particles. Lane²⁷ has written an excellent review article on the preparation of biological specimens.

Natelson and Sheid,²⁸ and Mathies and Lund²⁹ have contributed valuable work wherein they apply X-Ray



Projection Microradiography





X-ray Spectrograph

Spectroscopy to clinical laboratory problems. Natelson's paper deals with phosphorus and total blood iron as a measure of hemoglobin content. Mathies and Lund treat the subject of bromide (total bromide) in human blood, serum, urine, and tissues.

FOOTNOTES

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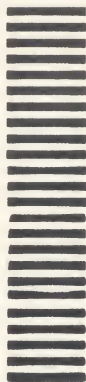
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